PA-10C

| QUERY CONTROL FORM | | | | RTIS USE ONLY | |
|---|---|---|---|---|---|
| Application No. | 09/724,876 | Prepared by | JB | Tracking Number | 5985922 |
| Examiner-GAU | Nashed - 1652 | Date | 9/23/04 | Week Date | 7/26/04 |
| | | No. of queries | 1 | IFW | |

| JACKET | | | |
|---|---|---|---|
| a. Serial No. | f. Foreign Priority | k. Print Claim(s) | p. PTO-1449 |
| b. Applicant(s) | g. Disclaimer | l. Print Fig. | q. PTOL-85b |
| c. Continuing Data | h. Microfiche Appendix | m. Searched Column | r. Abstract |
| d. PCT | i. Title | n. PTO-270/328 | s. Sheets/Figs |
| e. Domestic Priority | j. Claims Allowed | o. PTO-892 | t. Other |

**SPECIFICATION**

a. Page Missing
b. Text Continuity
c. Holes through Data
(d.) Other Missing Text
e. Illegible Text
f. Duplicate Text
g. Brief Description
h. Sequence Listing
i. Appendix
j. Amendments
k. Other

**CLAIMS**

a. Claim(s) Missing
b. Improper Dependency
c. Duplicate Numbers
d. Incorrect Numbering
e. Index Disagrees
f. Punctuation
g. Amendments
h. Bracketing
i. Missing Text
j. Duplicate Text
k. Other

**MESSAGE**

Amendment B9 (dated 7/23/04, Sc) attached has a blank Accession number.

Please resolve.

Thank you

initials JB

**RESPONSE**

initials

described in Wu and Kaiser, Dec. 1997, J. Bact. 179(24):7748-7758, was mixed with PCR primers Seq1 and Mxpil1 primers:

Seq1: 5'-AGCGGATAACAATTTCACACAGGAAACAGC-3' (SEQ ID NO:3); and

Mxpil1: 5'-TTAATTAAGAGAAGGTTGCAACGGGGGGC-3' (SEQ ID NO:4),

and amplified using standard PCR conditions to yield an ~800 bp fragment. This fragment was cleaved with restriction enzyme KpnI and ligated to the large KpnI-EcoRV restriction fragment of commercially available plasmid pLitmus 28 (New England Biolabs). The resulting circular DNA was designated plasmid pKOS35-71B. --

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Please replace the paragraph beginning at page 103, line 4, with the following rewritten paragraph:

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-- The sequence of the pilA promoter in these plasmids is shown below (SEQ ID NO:5). --

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Please replace the paragraph beginning at page 113, line 17, with the following rewritten paragraph:

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-- To improve production of epothilones from these vectors, the eryKS5 linker sequences were replaced by epothilone PKS gene coding sequences, and the vectors were introduced into *Streptomyces coelicolor* CH999. To amplify by PCR coding sequences from the *epoA* gene coding sequence, two oligonucleotides primers were used:
N39-73, 5'-GCTTAATTAAGGAGGACACATATGCCCGTCGTGGCGGATCGTCC-3' (SEQ ID NO:6); and N39-74, 5'-GCGGATCCTCGAATCACCGCCAATATC-3' (SEQ ID NO:7).
The template DNA was derived from cosmid pKOS35-70.8A3. The ~0.8 kb PCR product was digested with restriction enzymes PacI and BamHI and then ligated with the ~2.4 kb BamHI-NotI and the ~6.4 kb PacI-NotI restriction fragments of plasmid pKOS039-120 to construct plasmid pKOS039-136. To make the expression vector for the *epoA*, *epoB*, *epoC*, and *epoD* genes, the ~5 kb PacI-AvrII restriction fragment of plasmid pKOS039-136 was ligated with the ~50 kb PacI-AvrII restriction fragment of plasmid pKOS039-124 to construct the expression plasmid pKOS039-124R. Plasmid pKOS039-124R has been deposited with the ATCC under the terms of the Budapest Treaty and is available under accession number _____. --

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